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Co-products of the United States biofuels industry as alternative feed ingredients for aquaculture

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Chapter 23

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ABSTRACT

The tremendous growth of the biofuels industry has made large amounts of co-products (i.e. distillers grain and crude glycerin) available for use in aquafeeds. This chapter reviews the prospects and challenges associated with their use in aquafeeds. Properties of each product as it pertains to fish nutrition and available research are described for different fish species. Despite the apparent deficiency in lysine and the high fibre content in DDGS, considerable amounts of DDGS can be fed to omnivorous fish species without impact on growth or product quality. Nutrient variability is, however, an issue that needs to be considered when feeding DDGS to fish. The use of crude glycerin in fish is less clear, and further research is necessary before nutritional recommendations can be made.

INTRODUCTION

High energy prices and government policies that encourage the use of biofuels have spurred a tremendous growth in the ethanol and biodiesel industries, both in the United States and internationally over the last decade. In 2005, United States total ethanol production was estimated at 15.8 billion litres, and by early 2010, 51 billion litres of ethanol were produced (RFA, 2011). Similarly, biodiesel production has increased dramatically from 284 million litres in 2005 to 1.7 billion litres in 2007 (NBB, 2007). The surge in biofuel production has been simultaneously accompanied by a growing supply of co-products such as distillers grain and crude glycerin (i.e. glycerin or glycerol). Total supply of United States distillers grain was estimated at 32.9 million tonne in 2010, an increase of more than 13 fold compared with 2000 (Figure 1). The United States biodiesel industry is expected to produce an estimated 640 000 tonne of crude glycerin between 2006 and 2015 (Nilles, 2006). Excess glycerin in the market creates enormous marketing challenges and requires finding new uses for this co-product. Competitive pricing of low value crude glycerin has created opportunities for this co-product to be used in livestock feeding.

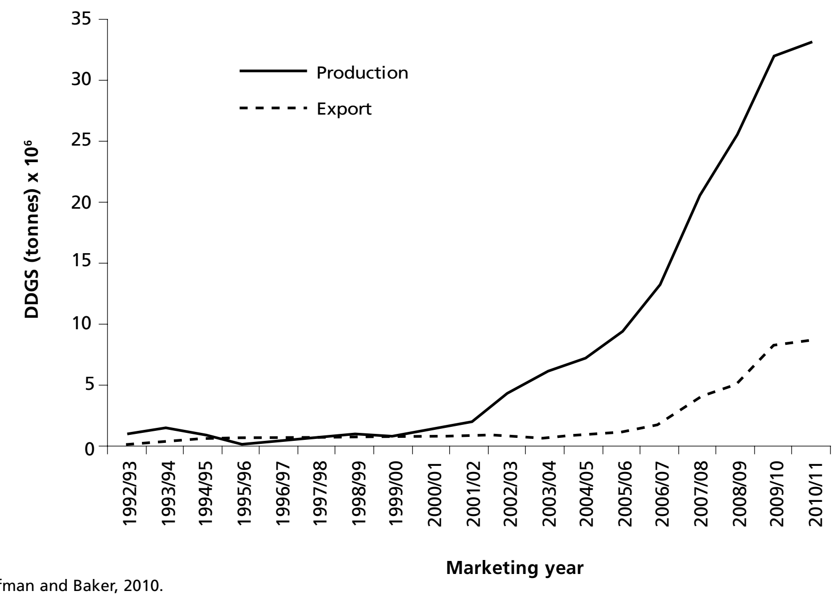
To date, distillers grain from the dry-grind ethanol industry have received considerable attention in animal feeds. In contrast, glycerol has been used more in industrial applications, although new research has shown that glycerol appears to be a promising energy source in animal diets. Distillers grain include traditional co-products, such as distillers wet grains, dried distillers grain with or without solubles (DDG and DDGS), and condensed distillers solubles (CDS). DDGS is the co-product that is most extensively produced in the ethanol industry. Recently, fractionation technologies used in ethanol production have resulted in new feeds with unique chemical compositions. Also, it is important to note that a small fraction of distillers grain is produced from beverage distilleries. However, the contribution of distillers grain from the beverage distilleries represented less than 2.7 percent of all the distillers grain produced in 2010/11 in the United States (Hoffman and Baker, 2010). In addition, maize (corn) is the primary feedstock grain used to make ethanol, accounting for more than 98 percent of all DDGS produced (Hoffman and Baker, 2010). Hereafter, the term "distillers grain" will refer to distillers dried grains with solubles (DDGS) (from maize) unless otherwise noted. Currently, DDGS is fed primarily to beef and dairy cattle, swine and poultry (Figure 2). No estimates on the current use of DDGS in aquafeeds could be found, but it is expected to be very small.

Another high growth sector in recent years has been aquaculture. Aquaculture has been growing at a rapid pace of approximately 6.2 percent per annum, from 38.9 million tonne in 2003 to 52.5 million tonne in 2008 (FAO, 2008), and currently accounts for over 50 percent of all food of aquatic origin consumed by humans worldwide. The value of aquaculture production was estimated at US$ 98.4 billion in 2008. However, concerns exist over the sustainability of aquaculture for a number of reasons, one of which is the increased pressure on feed ingredients, especially fishmeal

MAIN MESSAGES

- **DDGS from fuel ethanol production can be an effective protein ingredient in aquafeeds.**
- **DDGS serves to replace SBM and maize in the diet, but not fishmeal.**
- **For most fish species, a level of 20% DDGS appears to be the maximum inclusion if supplemental lysine is not added.**
- **If supplemental lysine is used, maximum DDGS levels greater than 20% can be used.**
- **Crude glycerine from biodiesel production appears to be a potential energy source.**
- **Much work needs to be conducted on use of glycerin in fish diets.**

FIGURE 1
Production and exports of distillers dried grains with solubles (DDGS) from the United States dry-grind fuel ethanol industry



Source: Adapted from Hoffman and Baker, 2010.

and fish oil. Fishmeal used in aquaculture represented 68.2 percent of total global fishmeal production in 2006 (Tacon and Metian, 2008), but increased pressure due to exploiting marine resources and rising prices could ultimately decrease the use of fishmeal, as it will inevitably be replaced by less expensive alternative proteins.

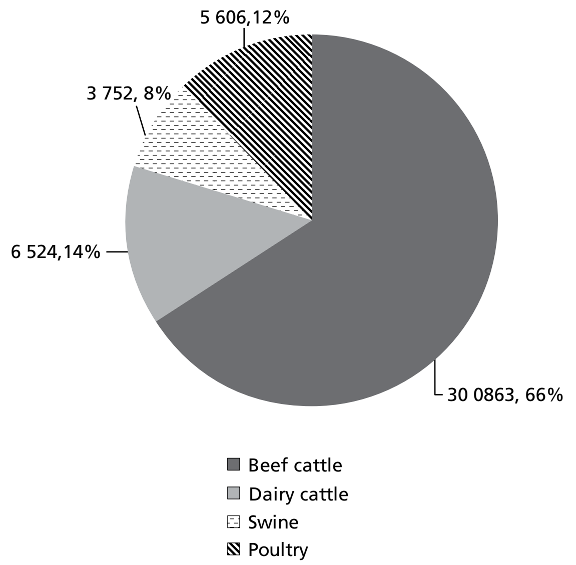
DDGS, a relatively cheap protein source (Figure 3) compared with fishmeal, is a candidate plant protein. During the last 10 years, DDGS market price has been generally between 5 percent and 20 percent that of fishmeal. While DDGS is not recommended as a direct, complete replacement for fishmeal, it can be used with, or in lieu of, other plant proteins (such as soybean meal – SBM) to reduce the use of fishmeal in aquafeeds. As shown in Figure 3, over the last decade the price of DDGS has ranged from approximately 20 percent to 70 percent that of SBM.

This chapter will review the nutrient composition of major biofuels (i.e. maize-based fuel ethanol and soy-based biodiesel) co-products (i.e. distillers grain and crude glycerin), will provide summaries of available nutritional studies for different fish species, and will conclude with final remarks on challenges associated with these co-products and areas of needed research.

Before proceeding, however, it is important to note a few key issues. First, maize is the primary feedstock for fuel ethanol production in the United States. Other starch-rich materials can theoretically also be used to produce ethanol, including barley, cassava, field peas, millet, triticale, oats, rice, rye, sorghum, sweet potato and wheat. Unfortunately, most of these alternative starch sources have only been investigated on a laboratory- or pilot-scale and are not readily commercially available. Not surprisingly, fish feeding trials are essentially non-existent for co-products from these substrates, and thus will not be discussed in this chapter.

In contrast, biodiesel can be produced from a variety of oilseeds and lipid-containing materials, including canola

FIGURE 2
Estimates of DDGS use by livestock class (in million tonnes) and percent of total for each use



Source: Adapted from Hoffman and Baker, 2010.

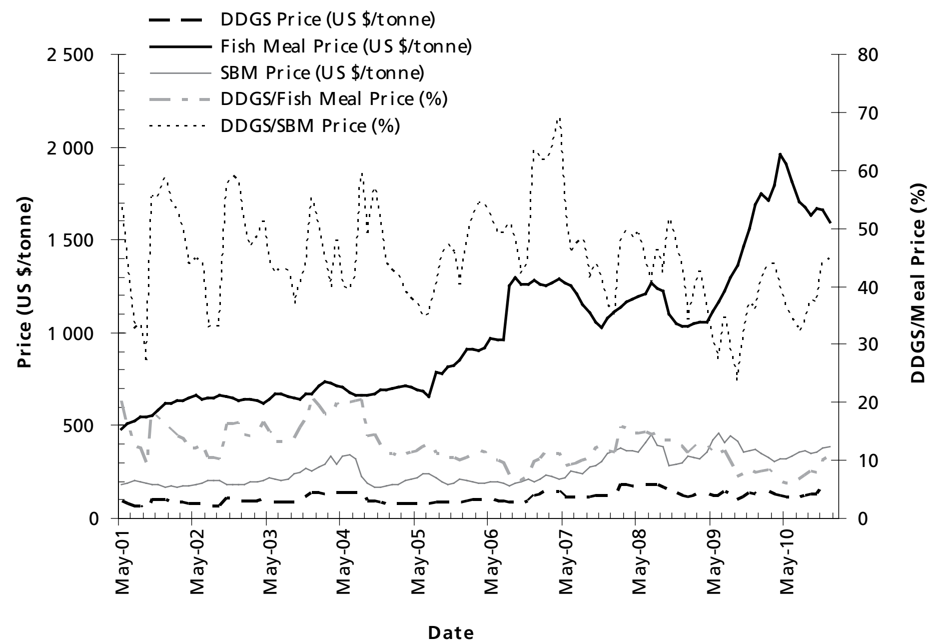
(rapeseed), castor beans, copra, cottonseed, flaxseed, jatropha, palm oil, poppy seed, safflower seed, sesame seed, shea nut, sunflower seed, animal rendering and others. The primary source for biodiesel in the United States, however, is soybean. Thus, our discussion will be focused on soy-based biodiesel co-products. But, our discussion will be limited to glycerin, and will not cover SBM or various soy protein concentrates or isolates. These topics have been covered in depth elsewhere (Gatlin *et al.*, 2007; Hertrampf and Piedad-Pascual, 2000; U.S. Soybean Export Council, 2011). Furthermore, algae-based biofuels have much promise for the future of the biofuels industry, but, to date, post-extraction algal residues use in any fish feeding trials has not been reported.

PROPERTIES OF DISTILLERS GRAIN

Physical properties

Some of the physical properties that are important to aquafeeds include particle size, bulk density and flowability. Because of the small size (<1.4 mm, on average) and variable size distribution of particles (Bhadra, Muthukumarappan and Rosentrater, 2009), handling DDGS can pose some logistical problems. Bulk density determines capacity of transport vessels and storage facilities. As with other properties, bulk density varies among DDGS sources and averages ~480 kg/m^3 (Rosentrater, 2006). For comparison, bulk densities of maize and SBM are 721 and 658 kg/m^3, respectively (Letsche, Lammers and Honeyman, 2009.). The low bulk density of DDGS translates into higher transportation costs. Variations in bulk density may be due to differences in particle size and to the amount of condensed distillers solubles (CDS) added back to distillers grain during manufacturing. Several factors affect the

FIGURE 3
Market prices (US$/tonne) for DDGS, fish¬meal and soybean meal (SBM) over the last 10 years, and the price ratio of DDGS to each of these meals over time



Source: Adapted from ERS, 2011 and IMF, 2011.

TABLE 1
Typical physical properties of distillers dried grains with solubles (DDGS)

Physical property	Mean (SD)	Range
Water activity (-)	0.53 (0.02)	
Bulk density (kg/m^3)	389.3 (24.1)	490–600
Angle of repose (°)	26.5 (1.8)	35.94–41.60
Colour Hunter L (-)	40.0 (1.6)	36.56–50.17
Colour Hunter a (-)	8.0 (0.4)	5.20–10.79
Colour Hunter b (-)	18.2 (0.9)	12.53–23.36

Notes: (-) denotes dimensionless quantities. *Sources*: Means (and Standard Deviations) from Rosentrater, 2006; Ranges from Bhadra, Muthukumarappan and Rosentrater, 2009, 2010.

flowability of DDGS, including particle size, soluble solid and fat contents (which are due to the CDS addition level), dryer temperature and moisture content at dryer exit (Ganesan, Muthukumarappan and Rosentrater, 2008). Manipulation of these properties and pelleting often improves the flowability of DDGS (Ganesan, Muthukumarappan and Rosentrater, 2008; Rosentrater, 2007).

The colour of DDGS can vary from golden yellow to dark brown. The amount of condensed distillers solubles (CDS) added to the distillers grain, the original colour of the feedstock grain and drying temperature are all factors that affect the colour of DDGS (Ganesan, Muthukumarappan and Rosentrater, 2008; Noll, Parsons and Walters, 2006). Colour can be a good indicator of heat damage (Maillard reaction) that occurs during the drying of distillers grain, especially to the most heat labile amino acid, lysine. In fact, the digestibility of lysine in swine has been shown to vary substantially among different DDGS sources (Stein *et al.*, 2006). In addition, a strong correlation has been determined between brightness/lightness (i.e. Hunter colour L) and digestible or bio-available lysine content of DDGS for poultry (Pahm *et al.,* 2009; Fastinger, Latshaw and Mahan, 2006) and swine (Pedersen, Pahm and Stein, 2005). Other physical characteristics of DDGS are summarized in Table 1.

Chemical properties

Nutrients in DDGS are concentrated nearly three times compared with those found in maize. This is because starch, which constitutes about two-thirds of the maize kernel, is removed during the fermentation process to produce ethanol. Predicting DDGS composition from that of maize, however, has to reflect multiple factors. Differences in processing within and among ethanol plants, especially drying conditions (temperature and time) and the amount of CDS added to the distillers grain and, to a lesser extent, the source and quality of maize, can create considerable variations in the nutrient composition of DDGS.

In fact, nutrient concentrations can vary substantially among DDGS sources (i.e. ethanol plants) (Table 2). Several papers on nutrient composition and influencing factors are available (Spiehs, Whitney and Shurson, 2002; Belyea, Rausch and Tumbleson, 2004; Belyea *et al.*, 2010). It is also important to recognize that nutrient composition of DDGS, as found in older publications (such as NRC, 1993), may no longer be applicable because DDGS from that generation was predominately made from alcohol beverage distilleries, not the newer fuel ethanol plants of today. In general, DDGS is a good source of energy and protein for various livestock animals. Fuel-based DDGS contains, on average, 11.0 percent moisture, 30.8 percent crude protein, 7.4 percent crude fibre, 11.2 percent crude fat and 5.5 percent residual starch (UMN, 2011).

Specifically, fish have requirements for amino acids rather than crude protein, per se. The amino acid profile of DDGS reflects that of maize, with lysine being the most

TABLE 2
Typical nutrient composition of distillers dried grains with solubles (DDGS)

Item	UMN	Spiehs, Whitney and Shurson, 2002	NRC
Dry matter (%, as is)	89.2 (1.6)	88.9 (1.7)	91.0
Crude fat	11.2 (14.3)	10.9 (7.8)	10.2
Crude fibre	7.4 (14.9)	8.8 (8.7)	10.0
Starch	5.5 (34.4)	-	-
Crude protein	30.8 (4.9)	30.2 (6.4)	29.7
Amino acids			
Arg	1.35 (9.6)	1.20 (9.1)	1.23
His	0.82 (9.1)	0.76 (7.8)	0.70
Ile	1.17 (7.1)	1.12 (8.7)	1.20
Leu	3.51 (8.8)	3.55 (6.4)	3.18
Lys	0.97 (12.6)	0.85 (17.3)	0.71
Met	0.60 (12.1)	0.55 (13.6)	0.55
Cys	0.61 (13.4)	-	0.51
Phe	1.49 (12.6)	1.47 (6.6)	1.53
Thr	1.12 (7.2)	1.13 (6.4)	1.08
Trp	0.23 (11.8)	0.25 (6.7)	0.11
Tyr	1.10 (12.6)	-	1.09
Val	1.55 (8.2)	1.50 (7.2)	1.65
Ash	5.7 (1.4)	5.8 (14.7)	7.0
Ca	0.05 (135.3)	0.06 (57.2)	0.15
P	0.79 (14.3)	0.89 (11.7)	0.73
K	1.02 (17.2)	0.94 (14.0)	0.44
Mg	0.31 (17.9)	0.33 (12.1)	0.18
S	0.69 (35.6)	0.47 (37.1)	0.38
Na	0.26 (188.8)	0.24 (70.5)	0.57
Cl	0.19 (25.5)	–	0.18
Zn (ppm)	58.80 (23.1)	97.5 (80.4)	87.91
Mn (ppm)	17.00 (26.1)	15.8 (32.7)	25.05
Cu (ppm)	6.00 (29.2)	5.9 (20.4)	58.02
Fe (ppm)	110.00 (39.1)	119.8 (41.1)	259.34
NFE	34.1	33.2	34.1

Notes: All nutrient values expressed as a percentage on a 100% dry matter basis (Coefficients of variation presented in parentheses when available); NFE= Nitrogen-free extract = 100 -(moisture + crude fibre + crude protein + crude fat + ash). *Sources*: UMN data are a compilation of data from 2008 and 2009 by University of Minnesota (UMN, 2011) (n=62). Spiehs, Whitney and Shurson (2002) is a compilation of data from 1997 to 1999 (n=118). NRC data are from Nutrient Requirements of Fish (NRC, 1993).

TABLE 3
Typical nutrient composition of other distillers grain products

Item	Wheat DDGS[1]	Triticale DDGS[2]	Sorghum DDGS[3]	De-oiled DDGS[4]	Maize HPDDG[5]	Sorghum HPDDG[6]
DM (%, as is)	90.9	89.4	88.4	87.5	91.4	92.3
Crude fat	5.7	–	10.8	3.5	4.0	3.2
Crude fibre	8.1	–	8.0	–	–	–
Starch	2.1	–	–	5.6	8.3	–
Crude protein	40.3	32.4	34.2	34.0	43.6	48.2
Amino acids						
Arg	1.65	1.45	–	1.59	1.70	1.85
His	0.82	0.75	–	1.04	1.17	1.11
Ile	1.37	1.17	1.41	1.47	1.79	2.18
Leu	2.67	2.51	4.44	4.26	5.99	5.89
Lys	0.89	0.78	1.01	1.09	1.28	1.73
Met	0.64	0.55	0.61	0.68	0.91	0.85
Phe	1.83	1.50	–	1.61	2.35	2.47
Thr	1.21	1.07	1.20	0.95	1.58	1.79
Trp	0.39	0.14	0.22	0.18	0.28	0.39
Val	1.78	1.49	1.86	1.43	2.25	2.63
Ash	5.7	–	4.5	5.3	2.1	5.0
Ca	0.18	–	–	0.06	0.04	0.13
P	1.05	–	–	0.84	0.45	0.82
NFE	31.1	–	42.8	–	–	–

Notes: DDGS = distillers dried grains with solubles; HPDDG = high protein distillers dried grain. All nutrient values expressed as a percentage on a 100% dry matter basis. NFE= Nitrogen-free extract = 100 -(moisture + crude fibre + crude protein + crude fat + ash). *Sources:* (1) Avelara *et al.*, 2010; Cozannet *et al.*, 2010; Oryschak *et al.*, 2010a; Bandegan *et al.,* 2009. (2) Oba *et al.*, 2010; Oryschak *et al.,* 2010b. (3) Jones *et al.*, 2010; Urriola *et al.,* 2009. (4) Mjoun *et al.*, 2010. (5) Jacela *et al.*, 2010; Mjoun *et al.*, 2010; Applegate *et al.*, 2009; Widmer *et al.,* 2008. (6) Jacela *et al.*, 2010.

limiting. Compared with SBM and fishmeal, DDGS supplies (on a crude protein basis) higher amounts of Met and Leu, similar amounts of His, Phe, Thr, Trp and Val, but lower amounts of Arg, Ile and Lys. When comparing the amino acid profile of DDGS with the requirements of tilapia and rainbow trout, it can be concluded that DDGS is deficient in lysine for both tilapia and rainbow trout, and in tryptophan for tilapia (Table 4). The imbalance of amino acids in DDGS can limit its value for fish when used as a sole protein source, although, when economically viable, synthetic amino acids can be used to correct deficiencies. Combining DDGS with other protein meals is another option. In addition, low digestibility of amino acids in DDGS may further limit its nutritional value in fish diets. It is, however, important to note that improvements in the protein quality of DDGS in terms of concentration and digestibility of amino acids from DDGS produced in new generation ethanol plants may be an indication of improved and more controlled production processes.

DDGS is also a good source of the vitamins niacin, riboflavin and vitamin E, as well as various minerals. DDGS contains high levels of P (0.80%), with the majority of this P being inorganic, making DDGS a good source of digestible P in chicks (Martinez Amezcua, Parsons and Noll, 2004) and swine (Pedersen, Boersma and Stein, 2007)**.** In contrast, DDGS contains low concentrations of Ca, Cl and other trace minerals. In addition, unlike most plant proteins, DDGS does not contain anti-nutritional factors, which can prove to be very problematic for some proteins. Variability in nutrient composition is still, however, an issue when dealing with DDGS. For this reason, access to reliable nutrient composition information is necessary to minimize risks associated with nutrient variation when DDGS is used in fish feeds.

As mentioned, the majority of United States distillers grain currently comes from dry-grind processing of maize into fuel ethanol, with smaller amounts being derived from sorghum (milo) and wheat, as well as a small percentage from beverage distilleries. In Canada, wheat and triticale represent the major grains used in ethanol production. Barley is another grain that can be used for ethanol production; however, lower ethanol yield and higher costs of production limit the use of barley in ethanol production. Moreover, barley DDGS has limited value in aquaculture feeds due to its residual content of beta-glucans. Likewise, recent changes aimed at increasing the efficiency of maize-based fuel ethanol production have resulted in a variety of distillers grain of different compositions, which are becoming available to the marketplace. The compositions of these other co-products that may have potential in aquaculture feeds are presented in Table 3. Although the chemical composition of some of these co-products appears attractive for use in aquafeeds, their nutritive value is still unknown. Except for limited information for high protein distillers dried grains (HPDDG), none of these co-products has been evaluated in fish diets. Research in monogastric species

TABLE 4
Ratio of essential amino acid supplies from different ingredients to the dietary requirements of different fish species

Amino acid	Tilapia			Rainbow Trout		
	DDGS	SBM	Fishmeal	DDGS	SBM	Fishmeal
Arginine	1.19	2.05	1.61	1.11	1.92	1.50
Histidine	1.77	1.77	1.50	1.44	1.44	1.22
Isoleucine	1.40	1.67	1.52	1.60	1.91	1.74
Leucine	3.84	2.62	2.34	3.09	2.11	1.89
Lysine	0.70	1.42	1.64	0.66	1.34	1.54
Methionine/cystine	1.39	1.01	1.27	1.49	1.08	1.36
Phenylalanine/tyrosine	1.74	1.75	1.39	1.78	1.79	1.42
Threonine	1.11	1.21	1.18	1.73	1.89	1.84
Tryptophan	0.87	1.63	1.15	1.44	2.71	1.91
Valine	2.06	1.85	2.05	1.59	1.43	1.58

Notes: DDGS = dried distillers grain with solubles; SBM = soybean meal. *Sources*: amino acid composition of DDGS from compilation of data from 2008 and 2009 by University of Minnesota (UMN, 2011) (n = 62); amino acid composition of SBM and fishmeal from Nutrient Requirements of Fish (NRC, 1993). Dietary requirements from NRC, 1993.

indicates lower amino acid digestibility of wheat and sorghum DDGS compared with the parent grain (Bandegan *et al.*, 2009) or maize DDGS (Urriola *et al.*, 2009; Jacela *et al.*, 2010; Oryschak *et al.*, 2010a).

Feeding value of distillers grain to fish

Fish performance

DDGS had been fed to fish for some time. In fact, the use of DDGS as component in aquafeeds can be traced back to the late 1940s (Phillips, 1949). Formal evaluations of DDGS began in earnest during the last two decades, and can be divided into two phases: prior to the ethanol boom (before 2000–2001), where most research primarily involved the use of co-products from the beverage alcohol/distillery industry (see, for example, Wu *et al.,* 1994, 1996a, b, 1997; Tidwell *et al.*, 2000); and post 2000–2001, where the majority of evaluated DDGS came from the fuel ethanol industry (see, for example, Shelby *et al.,* 2008; Abo-state *et al*., 2009; Schaeffer, Brown and Rosentrater, 2009; Schaeffer *et al.,* 2010). The chemical composition of DDGS produced from these two processes reflects the composition of the feedstock grain used. The distillery process usually uses a mixture of grains, including barley, rye, wheat and maize, while the fuel ethanol process primarily uses maize as the substrate for fermentation. Also, protein quality from the two processes may differ. As discussed previously, protein quality of DDGS has improved over time, resulting in a feed ingredient that is relatively consistent and highly digestible compared with older generation DDGS. Several factors control the amount of DDGS that can be effectively included in diet formulations for cultured fish. Those factors are related to species requirements and limitations imposed by the nutrient composition of DDGS. High fibre and unbalanced profile of amino acids in DDGS are the main constraints to including greater amounts in aquafeeds.

A summary of available data on feeding DDGS to various freshwater species is presented in Tables 5 and 6. These studies were essentially designed to test the incremental inclusion rate of DDGS, with the goal of establishing an optimal feeding rate. Most studies included a control diet where no DDGS was fed, allowing for direct assessment of the effect of DDGS on fish performance. To date, DDGS has been evaluated in 8 freshwater species, namely Nile tilapia (*Oreochromis niloticus*), channel catfish (*Ictalurus punctatus*), rainbow trout (*Oncorhynchus mykiss*), yellow perch (*Perca flavescens*), common carp (*Cyprinus carpio*), freshwater prawn (*Macrobrachium rosenbergii*), red claw crayfish (*Cherax quadricarinatus*) and sunshine bass (*Morone chrysops* × *M. saxatilis*), and two saltwater fish species: milk fish (*Chanos chanos*) and Pacific white shrimp (*Litopenaeus vannamei*). Tilapia and catfish have been the most studied species (Table 5). In many cases, DDGS was used as source of protein and energy, replacing maize meal and SBM at different proportions. DDGS also replaced other feedstuffs such as fishmeal, rice bran, wheat middlings, sorghum meal and meat and bone meal. It appears from the dataset that DDGS is generally accepted by the aforementioned species, with some differences. Tilapia and catfish have been shown to tolerate higher amounts of DDGS in their diets. In fact, feeding DDGS at levels as high as 60 and 70 percent DDGS, supplemented with lysine, resulted in optimal growth and feed efficiency of tilapia (Shelby *et al.,* 2008) and channel catfish (Webster, Tidwell and Yancey, 1991), respectively. In those species, DDGS can be fed at up to 30 percent of the diet without the need for supplemental lysine. For most species, an inclusion rate of 20 percent DDGS seems readily acceptable. Although, the inclusion of DDGS was restricted at 10 percent in studies involving Pacific white shrimp, red claw crayfish and sunshine bass, feeding more than 10 percent may be possible. Another way to improve the utilization of DDGS in fish diets may be achieved through taurine supplementation. It has been shown that taurine is conditionally indispensable in several fish species fed all-plant-protein diets. In fact, replacing fishmeal (which is a rich source of taurine), with plant proteins (which are usually very low in taurine), can result in taurine-deficient diets. Taurine supplementation has improved weight gain in several fish species, probably through enhanced voluntary feed intake (Takeuchi *et al.*, 2001; Park *et al.*, 2002; Gaylord, Teague and Barrows, 2006; Takagi *et al.*, 2006; Lunger *et al.*, 2007). It is expected that taurine supplementation for diets based on DDGS might improve feed utilization and growth of fish as well.

Tilapia

Published studies evaluating the use of DDGS in tilapia have involved a wide range of fish sizes (initial weight 0.5–190 g; final weight 6.1–907 g). In most studies, DDGS (0–100 percent) replaced maize and SBM in diets containing 0 to 8 percent fishmeal. Overall, feeding DDGS at levels between 15 and 30 percent appeared to maximize weight gain and feed efficiency. The addition of lysine allowed DDGS to be included at even higher levels of 40 to 60 percent. In general, feeding DDGS did not affect the flesh composition of tilapia.

Early studies on the use of DDGS in tilapia were conducted by Wu and colleagues using distillery-derived DDGS. Wu *et al.* (1994) reported that feeding 29 percent DDGS in combination with 6 percent fishmeal, or 22 percent DDGS in an all-plant-protein diet, to juvenile tilapia resulted in similar weight gain and feed conversion ratio as a control diet. Results from that study led Wu, Rosati and Brown (1996) to test whether higher inclusion rates of DDGS would sustain similar growth of tilapia compared with traditional diets. Two diets containing either 35 or 49 percent DDGS at dietary protein concentrations of 40 and 36 percent, respectively, were evaluated in tilapia fry. They found that the 35 percent DDGS diet resulted in similar weight gain and feed efficiency compared with the control diet, which was a 36 percent protein diet. Protein efficiency ratio was, however, higher in the control diet. At 49 percent DDGS, both weight gain and feed efficiency were depressed, indicating a lysine deficiency in diets containing the higher amounts of DDGS. Because lysine is the most limiting amino acid in DDGS-based diets, the addition of supplemental lysine may allow for greater DDGS inclusion levels. This question was investigated by Wu, Rosati and Brown (1997), who fed tilapia fry diets containing from 63 to 82 percent DDGS with added lysine. Overall, they found that, regardless of lysine supplementation, growth was negatively affected by high DDGS concentrations. In contrast, feed and protein efficiencies were similar for the 67 percent DDGS diet and the control diets.

In another study, Tidwell *et al.* (2000) evaluated the growth of juvenile tilapia fed diets consisting of pelleted or unpelleted DDGS (100 percent) in pond polyculture with freshwater prawn. Feeding either form of DDGS resulted in a 24 percent decrease in weight gain and 0.5 unit increase in feed conversion ratio compared with a commercial catfish diet. The economic efficiency (feed cost/weight gain), however, showed savings of US$ 0.29 and 0.40 per kg of fish produced, respectively, for pelleted and unpelleted DDGS, compared with the control diet.

To improve the dietary amino acid supply to the fish, one strategy is to feed DDGS as a blend with other proteins that are particularly rich in lysine. In this regard, Coyle *et al.* (2004) evaluated different protein blends in diets for juvenile hybrid tilapia. DDGS was included at 30 percent, with a combination of different protein sources, including fishmeal (8 percent), meat and bone meal (26 percent) and SBM (46 percent). They concluded that feeding DDGS with SBM resulted in lower weight gain and higher feed efficiency compared with the other protein combinations.

Additional studies (Lim *et al.*, 2007; Shelby *et al.,* 2008) evaluated the utilization of high levels of DDGS and whether supplementation with lysine would mitigate the associated negative effects on growth. Lim *et al.* (2007) found that optimal performance of juvenile tilapia was obtained at 20 percent DDGS without added lysine, while the addition of lysine to diets containing 40 percent DDGS improved feed utilization but not weight gain. Shelby *et al.* (2008), however, successfully included up to 60 percent DDGS with added lysine to diets containing 8 percent fishmeal, resulting in similar weight gain and feed efficiency compared with a control diet based on maize and SBM. These observations were confirmed by Abo-state, Tahoun and Hammouda (2009), who found that including up to 55 percent DDGS with added lysine in a 10 percent fishmeal diet resulted in even better weight gain and protein utilization by tilapia fingerlings compared with an SBM-based diet.

Recently, Schaeffer, Brown and Rosentrater (2009) found that weight gain, feed efficiency and fillet yield were adversely affected when DDGS was fed in excess of 30 percent of the diet, but their diets included no supplements. To more closely define the optimum inclusion rate for DDGS, Schaeffer *et al.* (2010) evaluated growth performance of juvenile tilapia fed diets with amounts of DDGS varying from 17.5 to 27.5 percent. They reported poorer growth of tilapia fed DDGS-based diets, and that feeding 20 percent DDGS resulted in maximum growth among the DDGS diets, although this corresponded to only 70 percent of that obtained with the commercial diet. The commercial diet contained 15 percent fishmeal, while the DDGS diets had 5 percent fishmeal.

It is clear that tilapia can effectively utilize DDGS; however, the large variability in the response of tilapia to feeding DDGS-based diets may indicate issues of consistency and quality of DDGS from different sources. Moreover, amino acid supplementation may be one way to improve the resulting performance of DDGS-based diets.

TABLE 5

Summary of studies evaluating the effects of feeding distillers grain products on growth performance, feed utilization and flesh composition in different fish species

Species	Fish weight (initial – final; g)	DDGS (%)	Ingredient(s) replaced	Trial duration (days)	Fishmeal (%)	Lysine[1] (%)	Optimum[2] (%)	Flesh composition	Reference[3]
Nile tilapia *Oreochromis niloticus*	34.9–67.7	0–27.5	Maize and SBM	55	5	no	17.5	–	Schaeffer *et al*., 2010.
	6.7–11	0–40	Maize and SBM	42	5	no	20	–	Schaeffer, Brown and Rosentrater, 2009.
	2–23	0–55	Maize and SBM	70	10	0–0.4	28/55	–	Abo-state, Tahoun and Hammouda, 2009.
	6.7–68.6	0–60	Maize and SBM	84	8	0.9	up to 60	–	Shelby *et al*., 2008.
	9.4–60.5	0–40	Maize and SBM	70	8	0–0.4	20/40	Whole body protein decreased at 40%	Lim *et al*., 2007.
	2.7–68.5	0–30	FM and SBM	70	0–8	no	30	No effect	Coyle *et al*., 2004.*
	26–120	0–100.00	–	84	0	no	–	No effect	Tidwell *et al*., 2000.
	0.5–11.4	0–82	CGF and SBM	56	0	0.25–0.75	none	–	Wu, Rosati and Brown, 1997.*
	0.4–20.9	0–49	Maize	56	0	no	35	–	Wu, Rosati and Brown, 1996.*
	30–122.4	19–29	Maize and SBM	103	0–6	no	29	–	Wu *et al*., 1994..
Hybrid tilapia *O. aureus × niloticus*	1.5–6.1	0–40	FM and wheat	90	3	0.4	Up to 40	–	US grains Council, 2007a.
Red tilapia	190–907	0–15	Maize and rice bran	120	0	no	Up to 15	No effect	US grains Council, 2006.
Channel Catfish, *Ictalurus punctatus*	9.1–80.4	0–30	Maize, SBM, wheat midds	56	5	0.3	30	Fillet protein decreased	Li, Oberle and Lucas, 2011.
	12.6–156.7	0–30	Maize and SBM	63	0	0.3–0.39	10/30[4]	Fillet fat increased, Protein decreased	Li *et al*, 2010a.
	86–491	0–30	Maize, SBM, wheat middlings	150	0	0.1–0.2	Up to 30	No effect	Zhou *et al*., 2010a.
	1.2–8.7	0–30	Maize, SBM, wheat middlings	56	0	0.2	30	–	Zhou *et al*., 2010b.
	13.3–67.1	0–40	Maize and SBM	84	8	0.4	40	Whole body fat increased	Lim, Yildirim-Aksoy and Klesius, 2009.
	48–1227	0–40	SBM and wheat midds	330	1	0.80–0.28	30/40	Fillet fat increased	Robinson and Li, 2008.
	33–226	0–30	Maize and SBM	110	8	no	30	No effect	Webster *et al*., 1993.*
	12.4–54.5	0–35[5]	FM and maize	84	0	0–0.4	35/35	–	Webster *et al*., 1992.*
	10–79.3	0–70	Maize and SBM	84	10	0–0.4	35/70	Whole body protein decreased and fat increased	Webster, Tidwell, and Yancey, 1991.*

TABLE 5 (Cont'd)

Species	Fish weight (initial – final; g)	DDGS (%)	Ingredient(s) replaced	Trial duration (days)	Fishmeal (%)	Lysine[1] (%)	Optimum[2] (%)	Flesh composition	Reference[3]
Rainbow trout *Oncorhynchus mykiss*	36.8–186.5	0–4[6]	SBM	84	31–33	no	4	–	Thiessen, Campbell and Tyler, 2003.
	49.8–96.2	0–22.5	In combination with CGM, replaced FM and wheat flour	42	7.5–22.5	0–1.23	15/22.5	Whole body fat decreased at 22.5% without Lys but not when Lys was added	Cheng and Hardy, 2004a.
	21–158.4	0–30	In combination with CGM, replaced FM and wheat flour	84	0	no	30[7]	Whole body protein decreased and fat increased	Stone *et al*., 2005.
Yellow perch *Perca flavescens*	19.1–54.3	0–50	SBM and Celufil	126	24	no	40	No effect	Schaeffer, Brown and Rosentrater, I2011..
Milkfish *Chanos chanos*	17.8–93.2	0–40	SBM, FM and wheat	–	2	0.3	20	–	US grains Council, 2007a.
Common carp *Cyprinus carpio*	41–168	0–15	SBM and rice bran	120	5	no	Up to 15	No effect	US grains Council, 2007b.
Freshwater prawn *Macrobrachium rosenbergii*	0.5–41.4	0–40	Maize, SBM, FM	105	0–7.5	no	40	–	Tidwell *et al*., 1993.*
Pacific white shrimp, *Litopenaeus vannamei*	0.45–25	0–10	Sorghum and FM	63	0	no	Up to 10	–	Roy *et al*, 2009.
Red claw crayfish *Cherax quadricarinatus*	0.12–4.2	0–10	FM	56	0	no	Up to 10	–	de Yta *et al*., 2012.
	5.75–62.3	0–30	In combination with other plant proteins, DDGS replaced FM	97	0	no	Up to 30	Tail muscle protein increased	Thompson *et al*., 2006.
Sunshine Bass *Morone chrysops* × *M. saxatilis*	15–69.7	0–10	Maize, SBM, MBM	56	0	no	Up to 10	No effect	Webster *et al*., 1999.*

Notes: DDGS = distillers dried grains with solubles; SBM = soybean meal; FM = fishmeal; CGM = maize gluten meal; MBM = meat and bone meal. (1) Lysine needed to achieve the optimal performance. (2) Optimum determined based on growth gain and feed efficiency as similar or superior to a Control diet. When two optimum concentrations are given, the highest value corresponds to optimum concentration when lysine was added. (3) * indicates DDGS from alcohol distilleries, not fuel-based DDGS. (4) 10% for distillers solubles or distillers solubles from maize endosperm; 30% for DDGS. (5) Included at fixed rate with varying SBM levels, both replacing fishmeal and maize. (6) Thin distillers solubles. (7) Pellets containing DDGS processed either by cold pelleting or extrusion were tested: 20% inclusion of DDGS with cold pelleting resulted in similar gain weight and lower feed efficiency as control, but the inclusion of DDGS at all levels resulted in inferior performances when the diets were extruded at 130 °C.

TABLE 6
Summary of studies evaluating further aspects of feeding DDGS in different fish species

Species	Key findings	Reference[c]
Nile tilapia *Oreochromis niloticus*	The addition of up to 150 mg/kg of phytase to a 28% DDGS diet increased weight gain and feed utilization at 75 mg/kg	Tahoun, Abo-State and Hammouda, 2009.
	Dietary DDGS, at levels of 0, 10, 20 and 40% in diets, had no effect on haematology, immune responses, or resistance of Nile tilapia to *S. iniae* infection.	Lim *et al.,* 2007.
	DDGS had no effect on immune function or disease resistance.	Shelby *et al.*, 2008.
Channel catfish *Ictalurus punctatus*	Fish fed 20–40% DDGS diets had increased total serum immunoglobulin, and those fed the 30% DDGS diet had significantly increased antibody titres 21 days following *E. ictaluri* challenge.	Lim, Yildirim-Aksoy and Klesius, 2009.
	Organoleptic evaluation of fillets indicated higher intensity of fat complex flavour for fish fed graded amounts of DDGS.	Webster *et al.,* 1993.
Rainbow trout *Oncorhynchus mykiss*	Fractionation of wheat DDGS using sieving increased digestibility of DM and energy nutrient content in rainbow trout.	Randall and Drew, 2010.
	Phytase supplementation in diets containing 15% DDGS improved digestibility of dry matter, fat and some minerals.	Cheng and Hardy, 2004b.
	Replacing 50% of fishmeal with SBM and 1.65 g MHA/kg in a diet containing 18.5% of DDGS improved weight gain, FCR and apparent retention of crude protein and phosphorus.	Cheng, Hardy and Blair, 2003.
Sunshine Bass *Morone chrysops* × *M. saxatilis*	Digestibility of dry matter and organic matter, but not protein and lipid, with DDGS diets were less than those with diets consisting of fish and SBM.	Thompson *et al.*, 2008.

Notes: MHA is a feed supplement which contains methionine; FCR = feed conversion ratio.

Channel catfish

In most channel catfish studies, DDGS was included in place of a combination of SBM and maize. These studies agreed that DDGS is highly acceptable for channel catfish at levels in excess of 30 percent. Also, supplementation with lysine or the presence of fishmeal, or a combination, further increased the potential for inclusion rate of DDGS up to 40 percent or even higher. Fillets from fish fed DDGS appeared to be relatively low in protein and high in fat content, reflecting the composition of DDGS.

Early studies in catfish reared in recirculating systems and floating cages (Webster, Tidwell and Yancey, 1991; Webster *et al.*, 1992, 1993) showed successful feeding of DDGS up to 35 percent, which could be increased to 70 percent in a diet that contained 10 percent fishmeal and supplemental lysine. Webster, Tidwell and Yancey (1991) demonstrated that a blend of DDGS and SBM could be used to replace all of the fishmeal in the diet of juvenile channel catfish. The efficacy of feeding high amounts of DDGS in pond or recirculating systems was confirmed in recent studies (Li *et al.*, 2010a; Li, Oberle and Lucas, 2011; Zhou *et al.,* 2010a, b; Lim, Yildirim-Aksoy and Klesius, 2009; Robinson and Li, 2008). From these studies it can be concluded that feeding DDGS at levels up to 35 percent with supplemental lysine is feasible in an all-plant-protein diet.

New fractionation techniques being used in the ethanol industry offer the aquafeed industry new opportunities as well as challenges. Novel co-products often contain high crude protein concentration, which makes them more suitable for aquafeeds. Li *et al.* (2010a) showed that feeding HPDDG and distillers solubles at 20 and 10 percent, respectively, as part of an all-plant-protein diet resulted in improved weight gain and feed efficiency.

Rainbow trout

It is thought that DDGS has limited nutritional value for salmonids because of its high content of non-nutritive components, such as non-starch polysaccharides (NSP) and pigments. Conversely, the few available studies (Stone *et al.,* 2005; Cheng and Hardy, 2004a) showed some success in feeding DDGS to rainbow trout. These studies have demonstrated that DDGS can partially replace fishmeal when fed with maize gluten meal (CGM) and supplemental lysine. More specifically, Stone *et al.* (2005) evaluated the effects of feeding DDGS (0–30 percent) and pellet processing method on growth and feed efficiency of rainbow trout. They found that when cold pelleting was used, weight gain was maintained up to 30 percent DDGS, but feed efficiency was depressed at all DDGS inclusion levels. In contrast, feeding DDGS resulted in inferior performances when the diets were extruded at 130 °C.

In another study, Cheng and Hardy (2004a) reported that 50 percent of the fishmeal could be replaced by feeding 15 percent DDGS with appropriate amounts of CGM. The inclusion rate was increased to 30 percent when the diets were supplemented with lysine.

On a different tack, Thiessen, Campbell and Tyler (2003) investigated the use of thin distillers solubles as a palatability enhancer in rainbow trout fed different proteins. The inclusion of 4 percent thin distillers solubles did not promote any additional appetite or growth of rainbow trout.

Other species

The value of DDGS in other species cannot yet be firmly established, since for most species, only one study can be found in the literature. Furthermore, in most cases, the use of DDGS was restricted to relatively low levels. In yellow perch, Schaeffer, Brown and Rosentrater (2011) reported that the inclusion of DDGS up 40 percent to partially replace SBM resulted in maximum growth and feed efficiency. Such performances were probably possible because of the high inclusion of fishmeal in those diets (24 percent). In sunshine bass, replacing maize meal with DDGS at 10 percent resulted in similar weight gain and feed efficiency (Webster *et al.,* 1999).

In its efforts to expand the use of DDGS in Asian aquaculture, the United States Grains Council led multiple experiments in major fish species grown in Asia, such as tilapia, common carp and milkfish. Farm studies have demonstrated that DDGS can be effectively fed at up to 15 and 20 percent, respectively, for common carp and for milkfish (U.S. Grains Council, 2006, 2007a, b).

Studies in crustacean species suggest that DDGS can be a viable source of protein to partially replace common protein sources such as fishmeal and SBM. Tidwell *et al.* (1993) evaluated the inclusion of 40 percent DDGS with SBM to partially or completely replace fishmeal for prawns grown in ponds. The fishmeal was reduced from 15 to 0 percent of the diet. Prawns fed DDGS diets had similar survival, yield per ha and feed conversion ratio compared with prawns fed the control diet with 15 percent fishmeal.

In Pacific white shrimp, Roy *et al.* (2009) reported similar weight gain, but lower biomass due to a tendency for higher mortalities for shrimps fed 10 percent DDGS compared with other feed alternatives, including poultry co-products, fishmeal and pea meal.

Two studies are available for red claw crayfish. Thompson *et al.* (2006) evaluated two levels of DDGS (18.3 and 30 percent) in diets with or without fishmeal. As DDGS increased in the diet, SBM increased and both sorghum and fishmeal decreased. They reported that feeding DDGS with SBM was equally effective in maintaining growth and feed efficiency as diets containing fishmeal. In another study, de Yta *et al.* (2012) fed the same dietary treatments previously evaluated for white Pacific shrimp by Roy *et al.* (2009) and found that similar to white shrimp, red crayfish can be fed a diet that contains 10 percent DDGS.

Flesh nutritional characteristics

Available data (Tables 5 and 6) suggest that feeding DDGS to various fish species is associated with alterations primarily in protein and fat contents of the final fish flesh. Feeding DDGS appears to increase fat content and decrease protein content, and these changes occurred either disjointedly or simultaneously (see, for example, Li, Oberle and Lucas, 2011; Li *et al*., 2010a; Lim, Yildirim-Aksoy and Klesius, 2009.; Robinson and Li, 2008; Lim *et al.,* 2007; Stone *et al.,* 2005; Cheng and Hardy, 2004a; Wu *et al*., 1996; Webster, Tidwell and Yancey, 1991). In other instances, the flesh composition remained unchanged (Schaeffer, Brown and Rosentrater, 2011; Tidwell *et al.*, 2000; Webster *et al.,* 1993, 1999). High fat concentrations and unbalanced amino acid profiles in the DDGS have been reflected in the flesh of fish fed DDGS-based diets. Thus, to mitigate some of these effects, dietary adjustments are necessary when feeding DDGS to various fish.

Organoleptic evaluations of fish fed DDGS-based diets are limited. Wu *et al.* (1996) found no differences in flavour characteristics of cooked tilapia, except a decline in "sweet" intensity for fish receiving 29 percent DDGS in their diets. Similarly, Webster *et al*. (1993) concluded that feeding DDGS to channel catfish had no adverse taste effects.

One of the concerns of feeding high amounts of DDGS is the negative impact on fillet pigmentation. DDGS contains, on average, 37 ppm of the xanthophyl pigments lutein and zeaxanthin, and this concentration varies among sources due to differences in heat treatment during drying of distillers grain (Salim, Kruk and Lee, 2010). Yellow pigments in DDGS will transfer to muscle tissues, which may render the final product less marketable. Li, Oberle and Lucas (2011) demonstrated that these pigments can be completely removed following the extraction of DDGS with ethanol. Feeding such products resulted in fillets with similar colouration to those from fish fed a SBM-based diet.

Digestibility

Digestibility coefficients are important for estimating the energy value and optimizing the use of ingredients in feeds. These are particularly important for co-products of the fuel ethanol industry, given the large variability associated with these materials. Evaluation of nutrient digestibility from DDGS in monogastric animals (swine) showed that digestibility of dry matter, energy, protein and lysine are low compared with traditional feedstuffs such as maize and SBM (Shurson, 2006; Stein *et al.*, 2006).

Information on DDGS nutrient digestibility in fish is rare. Thompson *et al.* (2008) compared nutrient digestibility from different feeds in sunshine bass. They reported very low digestibility coefficients for dry matter and organic matter (<15 percent) compared with SBM and fishmeal, which exceeded 40 and 60 percent, respectively. Protein and lipid digestibility for DDGS were 65 and 69 percent, respectively. Protein digestibility exceeded 84 percent for fishmeal and SBM, while lipid digestibility averaged 92 percent for fishmeal and 57 percent for SBM. Low digestibility of nutrients can increase faecal output and may deteriorate fish culture water quality. To date, there are no reports on nutrient digestibility from DDGS in omnivorous fish.

Fractionation techniques recently employed in ethanol production have resulted in new co-products, generally with higher protein, lower fibre and lower fat contents compared with traditional DDGS. Thus such products may have improved digestibility and nutritive values compared with conventional DDGS. For example, Randall and Drew (2010) evaluated the digestibility of nutrients from different fractions obtained by sieving of wheat DDGS. Sieving increased crude protein and decreased fibre concentrations. In addition, sieving improved digestibility of dry matter and gross energy, whereas digestibility of ether extract and protein were unaffected and high, exceeding 90 and 100 percent, respectively. The use of enzymes such as phytase can improve the nutritive value of the feed. Studies in poultry showed that in addition to improvement in phosphorus availability, supplementation with phytase improved protein and amino acid digestibility and availability in poultry diets (Rutherford *et al.*, 2004). Similarly, Cheng and Hardy (2004b) evaluated different doses of microbial phytase in the diets of rainbow trout that contained 30 percent DDGS. Improvement in dry matter, ether extract, Ca, Mg, phytate-P, total P, Mn, Cu and Zn were observed when adding phytase as low as 300 FTU (phytase units)/kg of diet. Protein digestibility from these diets was high and similar to the reference diet, averaging 90 percent. Recently, Tahoun, Abo-State and Hammouda (2009) showed that feed utilization was improved by the addition of 75 mg/kg of phytase to a 28 percent DDGS diet fed to Nile tilapia.

Immune function

DDGS contains approximately 3.9 percent yeast cell biomass (Ingledew, 1999). Yeast components such as beta-glucans, mannan-oligosaccharides, chitin, proteins, nucleotides, vitamins and trace minerals are important in modulating immune function. The potential of DDGS to stimulate immune function in fish is unclear. For example, feeding DDGS had no effect on immune function or resistance to bacterial infection in Nile tilapia (Shelby *et al.,* 2008; Lim *et al.,* 2007). In channel catfish subjected to *Edwardsiella ictaluri* challenge, feeding DDGS increased immunoglobulin, antibody titre and days to first mortality. Mortality was decreased, suggesting improved resistance to pathogen infection (Lim, Yildirim-Aksoy and Klesius, 2009). The authors suggested further investigation of the immunostimulatory effect of DDGS and the identification of potential active components that may be present in DDGS.

DISTILLERS GRAIN: ISSUES, CHALLENGES, KNOWLEDGE GAPS AND RESEARCH NEEDS

Overall, it appears that DDGS can be an effective source of energy and protein for fish. DDGS is not, however, recommended to be a direct, complete substitute for fishmeal or SBM. It is most effective when it replaces a combination of SBM and maize. Furthermore, the inclusion of DDGS is facilitated by the use of fishmeal and synthetic amino acids (primarily lysine) to improve the overall supply of amino acids to fish.

The use of DDGS in aquafeeds does present some challenges and limitations. Quality variation remains a major shortfall to using DDGS. Fish require high quality and dependable sources of nutrients to achieve high performance levels. DDGS can fill such requirements, provided the source is known, of consistent quality, and access to nutrient composition is available on a regular basis to nutritionists. DDGS also has some nutritional limitations when it is fed to fish. The high fibre content of DDGS, coupled with low digestibility of some nutrients, may limit its use in some fish species where nutrient-dense feeds are required. In addition, in recirculating aquaculture systems, DDGS use may also affect water quality because of potential increased faecal output.

When technical aspects are considered, handling of DDGS can pose some logistical problems because of the inherent physical properties of this granular bulk solid. Low bulk density and particle stickiness, which can lead to flowability problems, are the major challenges to the use of DDGS in animal feeds. These issues create transportation inefficiencies at all feed manufacturing levels, from transportation to feeding systems at the farm. These issues can be managed by implementing approaches such as manipulation of particle size and moisture content, or by the addition of flow agents. In aquaculture, feed is commonly manufactured using extrusion processing. Since DDGS contains high fibre and fat contents, coupled with a low starch level, extrusion of feeds containing DDGS can pose some difficulties. Once gelatinized (due to high processing temperatures), starch acts as a binder. Our research has shown that these limitations can be surmounted through the understanding of different interactions between process parameters and feed material. We have evaluated the extrusion of aquafeeds based on DDGS under a variety of processing conditions. Generally, as DDGS increases in the blend, decreases in pellet durability, expansion ratio, mass flow rate (throughput) and an increase in unit density and sinking velocity (i.e. no floatability) are observed. See, for example, Ayadi *et al.* (2011); Chevanan, Rosentrater and Muthukumarappan (2010); and Kannadhason *et al.,* (2010). Overall, it can be concluded that optimum pellets in terms of bulk density, durability and water stability can be obtained when DDGS is included at about 20 percent of the diet, which coincide with the optimum feeding level for most fish species. Improvements in pellet quality at high levels of DDGS are possible by the addition of different starches and binders.

Other challenges include mycotoxins, antibiotics and pigmentation. DDGS may contain mycotoxins if the parent grain is contaminated, although this risk is minimal in the Midwest United States, where most ethanol production plants are located. In addition, more ethanol plants have implemented stricter standards for grain selection. Again, knowing the source of DDGS and test results, especially during growth seasons with most risk of mycotoxin development, are important for safe utilization of DDGS. Recently, concerns about antimicrobial residues in DDGS have surfaced. Antimicrobials, such as penicillin, virginiamycin, erythromycin and others, are commonly added to the fermentors to control bacterial infections with the goal to optimize ethanol production by yeasts. These antibiotics can end up in the DDGS; however, it is believed that they will be completely deactivated under the extreme temperatures and pH conditions applied during ethanol production. In addition, heat treatments associated with extrusion cooking could further inhibit such substances. Thus, the issue of antimicrobials in DDGS, although serious, is more speculation rather than a reality in animal feeds. Pigmentation of tissues is also of concern when feeding DDGS, especially to salmonids. Feeding DDGS to salmonids is believed to alter flesh pigmentation from the typical pink colour to a less desirable yellowish colour, but to date there are no published studies evaluating the effect of DDGS on the pigmentation of fish tissues.

Finally, as the ethanol industry increases the efficiency of producing ethanol, different distillers co-products will become available, creating more challenges and opportunities for the aquafeeds industry. These products are expected to be more nutrient dense, as the fibre fraction can be further fermented and the fat extracted, leading to products composed mainly of protein and ash. Such products may be more compatible with fish requirements, but will need research to characterize them and assess their nutritional value and efficacy for different fish species.

PROPERTIES OF CRUDE GLYCERINE

The principal co-product of biodiesel production is crude glycerin. Common feedstocks used in the biodiesel industry include pure or waste vegetable oils, or a mixture, and rendered animal fats. Refining of crude glycerin is often limited to large scale biodiesel producers, which make high purity glycerol for applications in the food, pharmaceutical and cosmetic industries. Small-scale producers generally limit the purification process to the removal of excess alcohol to yield a low value co-product with limited uses (Thompson and He, 2006).

Physical and chemical properties

Crude glycerine contains impurities, including spent catalysts, residual methanol, methyl esters, oils and fats, soaps, free fatty acids and various minerals such as Ca, Na, Cl, K, Mg, P and S (Thompson and He, 2006; Dasari, 2007). Some of the physiochemical properties of crude glycerine are presented in Table 7. Considerable variation exists among crude glycerine sources, largely because of differences in the biodiesel production processes and the parent feedstock used. Mader (2011) showed that crude glycerine derived from animal fats contained less glycerol and more impurities than that derived from vegetable oil feedstocks. Common glycerol content is between 75 and 85 percent; however, glycerol content as low as 38.4 and as high as 96.5 percent of the total crude glycerine can be found on the market (Hansen *et al.*, 2009). Other major constituents are moisture, fat and a variety of minerals. Residual methanol is usually found at low concentration (<100 ppm); however, samples containing higher concentration (>15 percent) can be found, creating some health concerns when crude glycerine is fed to livestock (Hansen *et al.,* 2009). The USDA Food and Drug Administration (FDA) limits methanol content

TABLE 7
Physiochemical properties of crude glycerine

Item	n	Average	Min.	Max.	Reference
Pure glycerol (%)	39	78.58	38.4	96.5	1, 2, 3, 4, 5, 6, 7, 8, 9
Moisture (%)	27	8.20	0	24.37	4, 5, 6, 7, 8, 9, 10
Protein (%)	10	0.26	0.05	0.82	1, 3, 4, 5, 9
Fat (%)	11	5.54	0.12	15	1, 3, 4, 5, 10
Ash (%)	31	4.15	0	29.4	1, 3, 4, 5, 6, 7, 9
Na (%)	2	1.23	1.2	1.26	5, 9
Cl (%)	2	1.78	1.7	1.86	5, 9
GE (KJ/kg)	9	18340	15119	20510	1, 5, 10
pH	25	6.20	2	10.8	4, 5, 6, 7, 10
Methanol (%)	31	1.72	0.0009	14.99	2, 3, 4, 5, 6, 7, 8, 10
Density (g/cm^3)	11	1.20	1.07	1.26	6
Viscosity (4.45 °C, cSt)	6	60.00	82	38	10
Viscosity (40 °C, cSt)	2	8.60	8.8	8.46	1
Colour (c.u.)	2	7.25	3.5	11	10

Key to references: 1. Thompson and He, 2006; 2. Dasari, 2007; 3. Groesbeck *et al.,* 2008; 4. Lammers *et al.*, 2008a; 5. Lammers *et al.*, 2008b; 6. Hansen *et al.*, 2009; 7. Kerr *et al.,* 2009; 8. Mach, Bach and Devant, 2009; 9. Gunn *et al.*, 2010; 10. Mader, 2011.

TABLE 8
Summary of studies evaluating the effects of feeding glycerol to different fish species

Fish species	Glycerol inclusion	Ingredient replaced	Optimum (%)[a]	Flesh composition	Reference
Channel catfish (*Ictalurus punctatus*)	0-20	Maize	10	Fillet fat decreased	Li *et al*., 2010b[(1)]
Rainbow trout (*Oncorhynchus mykiss*)	0-12	Wheat middlings	–	No difference	Menton, Slinger and Hilton, 1986[(2)]

Notes: Optimum determined based on growth gain and feed efficiency as similar or superior to a Control diet. (1) Glycerol from biodiesel production. (2) Free glycerol included in low energy diets compared with a diet with similar energy density.

to 0.015 percent (150 ppm) in the final animal feed (FDA, 2006). Because the boiling point of methanol is 64.4 °C (Lide, 2001), it is believed that extrusion processing, commonly used in preparing fish feed, could eliminate any residual methanol found in crude glycerine. The colour of crude glycerine can range from clear to dark, reflecting pigments and compounds found in the parent feedstock.

Feeding value of crude glycerine to fish

There has apparently been only one published study (Table 8) that has evaluated the use of crude glycerine from the biodiesel industry in fish (Li *et al.*, 2010b). This study used crude glycerine as a source of energy to replace maize meal in the diet of channel catfish. They determined that a level of 10 percent was optimal for weight gain and feed efficiency; fillet fat content decreased at levels in excess of 5 percent. Pure glycerol was evaluated in another study in rainbow trout (Menton, Slinger and Hilton, 1986). Replacing wheat middlings by free glycerol up to 12 percent of the diet resulted in comparable weight gain, feed efficiency and carcass composition as fish fed a diet with similar energy density. The authors also found that glycerol can be an effective precursor for gluconeogenesis, but not for lipogenesis; however, rainbow trout cannot efficiently utilize glucose as a source of energy.

CRUDE GLYCERINE ISSUES, CHALLENGES, KNOWLEDGE GAPS AND RESEARCH NEEDS

Studies in other monogastric species suggest that crude glycerin can be a viable energy source. However, considering the current level of research in fish nutrition, which is essentially non-existent, an optimum level can not be recommended at this time. More studies are required to determine the efficacy of crude glycerol in major species such as tilapia, channel catfish, rainbow trout and yellow perch. As with other co-products, variability is an issue that hinders the use of crude glycerin in aquafeeds. Residual methanol is a potential safety hazard that needs to be addressed as well. Considering the physical characteristics of crude glycerin, other issues that should be evaluated include extrusion processing behaviour, handling and storage characteristics, potential corrosive effects, and the effect of feeding glycerin on flesh quality and health of fish.

CONCLUSIONS

DDGS and glycerine, co-products from the fuel ethanol and biodiesel industries, respectively, appear to be viable alternative feed ingredients for aquafeeds. DDGS is best used to replace a portion of SBM and maize in the diet. Because of variability issues and inherent nutritional limitations of DDGS, an inclusion level of up to 20 percent appears to be safe for most omnivorous fish species, whereas 10–15 percent is recommended for carnivorous fish such as rainbow trout. Specifically, DDGS can effectively be included at concentrations of 20 to 40 percent for channel catfish, tilapia and yellow perch, but at lower concentrations (10–15 percent) for rainbow trout, bass and some crustacean species. Nonetheless, when economically viable, supplementation with lysine will allow for higher DDGS inclusion rates. It has also been shown that DDGS can be included at high concentrations (up to 40 to 60 percent) while maintaining feed quality in terms of water stability and pellet durability when DDGS is part of extruded aquafeeds. Interestingly, the optimal inclusion level of DDGS in aquafeeds for superior pellet quality appears to be around 20 percent, which coincides with optimal fish performance in most species. In some species, nutritional characteristics of the final products can be altered. Lower protein and higher fat contents are usually observed when feeding DDGS above optimal levels. The efficacy of crude glycerine in fish diets is less evident. Very limited information suggests that glycerine might be used as an energy source. However, comprehensive investigation is still needed to address the use of glycerol as a feed ingredient for major fish species. The effect of glycerol on feed processing, final product quality, metabolism and health of fish are some areas that needs further research before glycerine can be efficiently and safely used in aquafeeds. Biofuels will clearly continue to play a key role in the global energy portfolio over the coming years, and co-products such as DDGS, glycerine, as well as other new co-products yet to be developed, will continue to grow in quantity. Aquafeeds may be a viable opportunity for their utilization.

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